Type 2 Diabetes and its Effects on MicroRNA-155 and MicroRNA-133a Expression in Lung Tissue of Male Rats

Maria Khezri

Tabriz Medical University: Tabriz University of Medical Sciences

Reza Rahbarghazi

Tabriz University of Medical Sciences Faculty of Medicine

Mahdi Ahmadi

Tabriz University of Medical Sciences

Siamak Sandoghchian

Tabriz University of Medical Sciences Faculty of Medicine

Alireza Nourazarian

Islamic Azad University Khoy

behrouz shademan

Ege University Faculty of Medicine: Ege Universitesi Tıp Fakültesi

Meysam Abdi

Tabriz University: University of Tabriz

Fatemeh khaki-khatibi (✉ Khzanfrjy@gmail.com)

Tabriz University of Medical Sciences

Short report

Keywords: Type 2 diabetes, lung disease, miRNA-133a, miRNA-155

Posted Date: October 11th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-952437/v1>

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Abstract

Background: The presence of an extensive vascular extent and large amounts of collagen and elastin in lung tissue make the lung parenchyma vulnerable to damage in diabetes. However, there are few studies on the pathophysiological effects of diabetes on lung tissue. In this study, we investigated the effects of type 2 diabetes on lung tissue pathology and the expression of miRNA-155 and miRNA-133a in lung tissue of male rats.

Methods: In this study, 14 male rats were divided into a control group and a group with diabetes. The group with diabetes received a high-fat diet and a single dose of streptozotocin for one month. After induction of T2D, the rats received a normal diet for the next four weeks. At week 8, the rats were euthanized, and lung tissue was collected to measure microRNAs and examine tissue changes.

Results: When lung tissue sections from diabetic rats were examined, the normal structure of the alveoli and alveolar sacs and bronchioles was disturbed. The thickness of interalveolar septa was increased due to the infiltration of inflammatory cells. The extensive alveolar collapse was the main cause of lung tissue structure disruption, and the accumulation of inflammatory cells and exudate secretions resulted in an interstitial pneumonia-like appearance. The expression of miRNA-155 was increased, and the expression of miRNA-133a was decreased in the lungs of diabetic rats compared with control rats.

Conclusion: We found significant changes in the lung tissue of diabetic rats. By studying changes in the expression of microRNAs in diabetes, they can be a diagnostic and therapeutic biomarker in the lungs of patients with diabetes.

Introduction

Diabetes mellitus refers to several metabolic disorders, all characterized by chronic hyperglycemia. In this disease, the ability to produce the hormone insulin is lost (type 1), or the body's cells become resistant to insulin (type 2). When blood sugar levels increase and affect metabolism, complications of the disease become noticeable [1, 2]. Cardiovascular, kidney and eye complications, problems of the nervous system, disorders of peripheral organs due to nerve damage and finally, heart and ischemic stroke are complications of diabetes in the body [3]. Diabetes is the seventh most common disease in the world in terms of economic and social burden. According to the World Health Organization, more than 488 million people were diagnosed with diabetes in 2014, and more than 1.6 million deaths were directly attributable to diabetes in 2016 [4].

Lung tissue is highly prone to damage and microvascular complications in diabetes because of its large vascular extent and abundant connective tissue [5]. Decreased lung function in people with diabetes is directly related to blood glucose levels, duration of diabetes, and severity of nonsmoking disease and obesity [6].

High blood glucose levels and the duration of the disease also increase the clinical symptoms of chronic lung disease [7]. Changes in the lung tissue of patients with diabetes include 1) an increase in the thickness of the basement membrane, which is due to an inflammatory process in the lungs of a patient with diabetes. 2) neutrophil infiltration and accumulation, and an increase in alveolar wall thickness, and 3) fibrosis toward inflammatory cell infiltration, as seen in hematoxylin and eosin stains [8, 9]. The effects of diabetes on the lungs and pulmonary disease have been demonstrated in many clinical studies. However, the pathophysiology and mechanism of the effects of diabetes on pulmonary problems are still largely unknown and unclear. Previous studies have shown pathologic changes in the lung tissue of patients with diabetes, but the exact mechanism of these changes is unknown [6, 8, 10].

MicroRNAs (miRNA) are small, uncoded, protected RNAs 18–25 nucleotides in length that control gene expression after transcription by inhibiting translation or initiating degradation of mRNAs [11]. It has also been shown that each miRNA has hundreds of target genes. Thus, even if the expression of a miRNA is changed, it has a significant impact on regulating target genes [12]. Their expression may be a marker of tissue health or disease. These miRNAs can be used as potential biomarkers for diagnosis, prognosis, and treatment [13].

To date, the involvement of many miRNAs in diabetic complications such as retinopathy, nephropathy, neuropathy, and cardiovascular and pulmonary complications has been demonstrated [14, 12]. Also decrease the expression of m miRNA-155 in severe asthma and its inhibition in people with lung inflammation and ductal mucus secretion. It decreases the number and secretion of Th2 lymphocytes. In asthma, the level of miRNA-133a also decreases. Increasing the expression of this miRNA decreases airway remodeling (by affecting airway smooth muscle) [15, 16]. MiRNA-133a expression is decreased in the bronchial smooth muscle of asthma patients, which increases RhoA levels and improves bronchial response and sensitivity [17]. MiRNA-133a plays an antifibrotic role in idiopathic pulmonary fibrosis (IPF) disease and inhibits differentiation by inhibiting TGF- β . Myofibroblasts and prevention of pulmonary fibrosis [18].

The level of miRNA-155 in the serum of patients with type 2 diabetes is low, suggesting that miRNA-155 controls blood glucose levels in patients with diabetes. Genetically modified mice expressing higher levels of miRNA-155 were more to have hypoglycemia and increased insulin sensitivity. miRNA-155 causes higher glucose uptake in all cell types [19, 20]. The level of miRNA-155 in foot ulcers of study participants in the diabetes group is higher than usual, which suppresses FGF7, effective in wound healing and wound epithelialization. By inhibiting miRNA-155, the wound healing rate was significant [20]. Studies on the inhibition of miRNA in preclinical models of asthma, cystic fibrosis (CF), and IPF have shown that therapeutic targets are satisfactory [21–23]. In animals, these miRNA inhibitors are administered via sprays and nebulizers, and this is good news for the search for a new way to treat chronic lung diseases [24].

Glycosylated protein in the lung and thoracic tissues after hyperglycemia, the resistance of collagen to proteolysis, and finally, the accumulation of this protein in lung connective tissue may cause lung disease

in patients with diabetes. Understanding the mechanism of these disorders will help diagnose and treat the lung damage caused by diabetes. MiRNAs are markers for various diseases. Therefore, in this study, we investigated the effects of type 2 diabetes on lung tissue damage and the expression of miRNA-155 and miRNA-133a in lung tissue of male rats.

Methods

Animals studied

This study approved by the ethics committee of the Tabriz University of Medical Sciences, with code 1398/344. In this study, 14 male Wistar rats (8-10 weeks) weighing 220–250 g were transferred after purchase to Animal Care Center from Tabriz University of Medical Sciences. The animals were kept in standard cages on a 12:12 dark-light cycle at temperatures ranging from 18 to 22°C, with free access to water and food. Ten days after transfection (to relieve stress and acclimate to the new environment), rats were randomly divided into two groups: Control (rats with a normal diet) and diabetic (rats with diabetes induction). The study site was the Applied-Drug Research Center of Tabriz University of Medical Sciences.

Induction of diabetes and Approval of diabetic rats

T2D was induced by a HFD (22% fat, 20% protein, and 48% carbohydrate) for four weeks, followed by intraperitoneal injection of a single dose of STZ (35 mg/kg body weight) [25]. To dissolve streptozotocin (STZ), 0.1 M cold citrate buffer with a pH = 4.5 was used. The diabetic and control rats received a normal diet for four weeks after the onset of diabetes. Before being killed, the diabetic and control rats were required to fast for 12 h. A glucose tolerance test (GTT) was then performed by administering 1 g/kg glucose to the diabetic and control rats. Blood glucose levels were measured after 0, 30 min, 60 min, 90 min, and 120 min using a rat glucose meter.

Preparation of lung tissue for analysis of tissue sections and expression of miRNAs

On the last day of week 8, lung tissue was collected for the measurement of miRNAs after animals were anesthetized by intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg) to assess tissue changes. Lung tissue was washed with cold, sterile normal saline. Then, one lung was frozen to examine gene expression, and the other was fixed in 10% formalin solution to examine tissue changes. The hematoxylin-eosin (H&E) method was used to evaluate the extent of lung injury. The histopathological examination included tissue changes such as leukocyte infiltration, perivascular edema, inflammation, and pulmonary fibrosis. Histopathological changes were examined using a light microscope (Olympus CH30 made in Japan) and photographed with an Olympus DP12 camera, U-TV0.5XC-2 Japan.

MicroRNAs extraction, cDNA synthesis and real-time polymerase chain reaction

Real-time polymerase chain reaction (PCR) was used to determine the transcription of miRNA-155 and miRNA-133a. For this purpose, lung tissue was rapidly sectioned, and RNA content was extracted using TRIzol (Roche, Germany) according to the manufacturer's instructions. A Picodrop 1000 spectrophotometer (Thermo Scientific, USA) was used to determine RNA concentration and integrity. Table 1 shows the sequences of all primers used to detect miRNA-155, miRNA-133a, and U6-6p. cDNA Synthesis Kit was used to generate cDNA (TaKaRa). Real-time PCR was performed on a Rotor-Gene 6000 instrument using a cocktail of cDNA sample (1L), SYBR Green Master Mix (5L; TAKARA), DEPC water (3.7L), and primers (0.3L) (Corbett, Australia). The PCR products were standardized to U6. The $2\Delta Ct$ technique was used to determine the relative quantitative expression of miRNA.

Statistical analysis

Analysis was performed using SPSS 19 software. All quantitative data were presented as mean $\pm$ SEM and analyzed with the student t-test. In this study, a value of $P < 0.05$ was considered significant.

Results

GTT confirms type 2 diabetes.

Because we autopsied the rats one month after they developed diabetes, we performed a GTT to confirm that the rats continued to have diabetes. GTT showed that glucose tolerance was impaired in the group with diabetes compared with the control group at week 8 (Figure 1).

The effect of diabetes on lung tissue

The lungs of both diabetic and control groups were examined by light microscopy with hematoxylin and eosin staining. The lung tissue sections of the rats in the control group showed normal tissue structure. Normal alveoli with the septum between the thin alveoli and alveolar sacs were visible. The alveoli walls were lined with type 1 and type 2 pneumocytes, each with its characteristics (type 1 pneumocytes are apartments and type II pneumocytes have large round domes) (Figure 2A).

When we studied the lung tissue sections of the diabetic rats, we observed the disruption of the natural structure of the alveoli and sacs and the bronchioles and interstitial vessels. The interstitial septa were thickened, mainly due to infiltration of inflammatory cells, including macrophages. We also saw an increase in the thickness of the epithelial layer covering the surface of the bronchioles. In some tissue sections, homogeneous material accumulated in the arteries, and we also noted exudative secretions between the alveoli. One of the most common findings on most slides and tissue sections was an extensive alveolar collapse, the leading cause of disruption of normal lung tissue structure. The accumulation of inflammatory cells and exudative secretions resembled interstitial pneumonia (Figure 2B).

The effect of diabetes on the expression of MiRNA-155 and MiRNA-133a in the lungs of rats

According to the results, the expression level of the miRNA-133a gene was lower in diabetic rats than in control rats (Figure 3A) ($p < 0.05$). However, the expression level of the miRNA-155a gene was higher in diabetic rats than in control rats (Figure 3B). ($p < 0.01$).

Discussion

The prevalence of diabetes is increasing every day. Therefore, study its adverse effects, including the effects of diabetes on patients' lungs. Previous studies have demonstrated pulmonary changes in patients with diabetes [26–28]. We studied the lung tissue of rats to control the pulmonary changes in diabetes. In this study, we investigated the effects of diabetes on the expression of two genes, miRNA-155 and miRNA-133a, in the lung tissue of diabetic rats.

In a study on the effects of diabetes on lung function, two groups of diabetic and non-diabetic overweight patients were examined. According to studies, all lung capacities (FVC, FEV1, DLCO, TLC) of patients with diabetes were lower than those of non-diabetic patients [29, 30]. In a study to evaluate the histopathological changes in the lungs of diabetic rats, changes in lung tissue were investigated in two groups: the group with diabetes and the control group. It was found that a significant increase in interalveolar septa was observed after four weeks, indicating cellular infiltration.

Some bronchioles showed patchy hyperplasia of the lower epithelium. Eight weeks after the onset of diabetes, an accumulation of alveolar macrophages and inflammatory cells was observed in the lung tissue, which increased the thickness of the septum between the alveoli and caused many alveoli to collapse, disrupting the normal tissue structure. The normal tissue structure was disturbed. Obstruction and thickening of the vessels occurred with exudate of fluid and blood into the lung tissue. Collagen fiber accumulation was also increased in the group with diabetes compared with the control group [31]. In our study, the lungs of both the control and groups with diabetes were examined. We observed significant changes in the lung tissue of diabetic rats compared with the control group. Increased thickness of interalveolar septa, infiltration of inflammatory cells, exudative secretions in the interstitial space, disruption of the typical appearance of normal lung tissue due to the collapse of alveoli and bronchioles, and similar occurrence of interstitial pneumonia are the sum of our findings from the study of lung tissue sections from diabetic rats.

According to searches in databases and articles, miRNA-133a has not been studied in the lungs of patients with diabetes. Also, the role of miRNA in type 2 diabetes has rarely been discussed. This miRNA is highly expressed in cardiomyocytes. According to a study by Shali Chen *et al.* [32] the expression of this miRNA was decreased in diabetic rats with cardiac fibrosis.

Expression of miRNA-133a in bronchial smooth muscle is markedly reduced in asthma patients, which increase RhoA levels and enhances bronchial responsiveness and sensitivity [15]. MiRNA-133a plays an antifibrotic role in IPF by inhibiting myofibroblast differentiation and preventing lung fibrosis by inhibiting TGF- β [18]. In our study, miRNA-133a expression was lower in diabetic rats than in controls, consistent with studies in diabetes. Because miRNA-133a in the lungs of patients has been little studied, our study

may represent a turning point in the study of miRNA-133a in the lungs of study participants in the diabetes group and other diseases. In our study, the expression of miRNA-155 was increased in the lungs of diabetic rats compared with control rats. A study investigating the effect of miRNA-155 on the pathogenesis of diabetes complications found that the expression of miRNA-155 was reduced in tissues other than the liver, including peripheral blood, heart, liver, sciatica, and aorta of diabetic rats. In this article, they also confirmed changes in the expression of this miRNA in diabetic rats [33]. In another study, the expression of miRNA-155 was also lower in diabetic rats [34]. However, these studies did not examine lung tissue. Thus, their results contradict our results, but they support the importance of this miRNA in diabetes.

A study by Dong Zhang *et al.* also showed that a deficiency of miRNA-155 plays a protective role in preventing cardiac fibrosis in diabetic rats [35]. In another study, a group concluded that miRNA-155 reduced the prophylactic effect of TGF- β [36]. In a study by Kuroski *et al.* [37] miRNA-155 increased collagen deposition and the production of TGF- β , which mediates pulmonary fibrosis. And Moses mentions that miRNA-155 increased collagen production mediated by TGF- β [38]. miRNA-155 plays a vital role in the progression of systemic sclerosis. Its expression is markedly increased in the lungs of patients with systemic sclerosis, and miRNA-155 is an essential biomarker for the progression of pulmonary fibrosis in these patients. This effect could be an important target for treating this disease [39]. And high levels of this miRNA are used in the diagnosis and severity of asthma. It is directly related to IL-13 level and negatively correlates with FEC and FEV1 [40].

In non-SCC lung cancer, we have a high expression of miRNA-155, which increases disease expression [41] and is essential because one of the significant lung changes in diabetes is lung fibrosis and increased collagen production. We also obtained the same result in this study. Additionally, all these articles agree with our study in which an increase in miRNA-155 was found. Although we did not investigate the exact pathogenesis of miRNA-155 expression in diabetic lung lesions, previous studies suggest that miRNA-155 cause fibrosis and increased collagen deposition by acting on inflammatory and fibrotic factors. According to recent articles, using miRNAs as diagnostic biomarkers and therapeutic targets is strongly recommended. These miRNAs could be a good target for the treatment and, more importantly, preventing diabetic lung problems.

Conclusion

The prevalence of diabetes is increasing every day. Therefore, research into its side effects, including the effects of diabetes on patients' lungs, is essential. In this study, we found changes in the lung tissue of diabetic rats. We also found that the expression of miRNA-155 increased in the lungs of diabetic rats, and the expression of miRNA-133a decreased in the lungs of diabetic rats. Based on all these findings, we suggest that miRNA-155 is a significant cause of lung injury in diabetes when its expression is increased and that miRNA-133a can cause lung injury when its expression decreases. To further support these findings, we recommend conducting studies with more extensive samples and investigating changes in lung tissue by manipulating the expression of these genes to evaluate their therapeutic or prophylactic

effects. We also recommend that future studies investigate the effects of diabetes on the expression of genes and miRNAs at the proteomics level.

Declarations

Acknowledgments

The authors thank Tabriz University of Medical Sciences for their financial support. We also thank the staff of the Stem Cell Research Center for their cooperation and advice.

Authors' contributions

MK: Data curation, Methodology, Writing – original draft. **MA, SS,** and **MA:** Methodology, Formal analysis. **AN, RR,** and **BS:** Conceptualization, Formal analysis, Writing – review & editing. **FK:** Conceptualization, Supervision, Project administration, Writing – review & editing. All authors read and approved the final manuscript.

Funding

This study was supported by a grant from Tabriz University of Medical Sciences (IR.TBZMED.VCR.REC.1398.344).

Availability of data and materials

The data and materials used in this study are available.

Compliance with ethical standards

Conflict of interest

The authors declare that there are no conflicts of interest

Ethical approval

The Ethics Committee of the Tabriz University of Medical Sciences approved the study protocols following the Declaration of Helsinki.

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Figures

Figure 1

Shows the GTT test in the control group and the groups with diabetes ($n = 7$) for each group. Bars represent means $\pm$ SEM.

Figure 2

lung tissue of control (A); histological examination of lung tissue in control group rats with normal alveolar structure and lung tissue of diabetic rats (B); View of histopathological changes of lung tissue in diabetic rats-H & E 100 stains.

Figure 3

Expression levels of miRNA-133a and miRNA-155 in lungs of control and groups with diabetes (for each group, n=7). Bars represent mean $\pm$ SEM. Statistical differences between the control and groups with diabetes: ++, $p < 0.05$ and +++, $p < 0.01$, respectively.